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I. V79 INHIBITION OF METABOLIC COOPERATION (IMC) ASSAY

The IMC testing of CSC obtained from the model cigarette sample X6D5BBL (BBL) was completed this month (1). All experiments showed a dose-related increase in activity in the range of 5-10 ug/ml. Statistical analysis indicated that the activity of BBL CSC was slightly higher than that obtained with the Kentucky Reference (2R1) CSC.

A series of experiments designed to define the activity of hydroquinone (HQ), a metabolite of phenol, were completed (2). Because of the inconsistent response of HQ in the IMC assay additional experiments were needed to obtain a definitive answer. Statistical evaluation indicated that HQ was moderately active in the IMC assay (slope = 16.3 % recovery/ug of agent/ml of treatment medium/3 day treatment).

To study the multistage effect(s) of promoters in the IMC assay, the activity of mezerein, a stage II promoter (3), was investigated. Although no toxicity was observed in the dose range (0.01-5ug/ml) tested, mezerein appears to be highly active. These preliminary results indicated that mezerein was as effective in inhibiting metabolic cooperation as the positive control, TPA. Experiments are being conducted to confirm these results, and the data will be reported next month.

A study was initiated to determine if CSC would inhibit the activity of a strong promoter (TPA) (1). Several doses of CSC, obtained from the Kentucky Reference cigarette (2R1), was tested simultaneously with one dose of TPA. In the absence of TPA, 2R1 CSC showed a dose-related increase in activity similar to that obtained previously. When both compounds were tested a significant increase in activity was observed in a dose-dependent manner when compared to TPA or 2R1 CSC tested alone. Experiments are now in progress to confirm and expand these findings.

To facilitate flow cytometric analysis of IMC results an experiment was conducted to evaluate the cytotoxicity of carboxyfluorescein diacetate (CFDA) (4). The results indicated that CFDA was not toxic throughout the dose range (0.1-100 ug/ml) tested. Experiments are now underway to test the effect of CFDA on metabolic cooperation.

II. CELLULASE TREATMENT OF TOBACCO

Two anaytical procedures have been implemented and standardized to facilitate examination of cellulase activity (4). They include the hexokinase mediated assay for glucose concentrations and dinitrosalicylic acid assay for free reducing sugars. In addition, an assay to quantitate the endoglucanase activity in cellulase is currently being evaluated.

III. REFERENCES

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4. Davies, B. D. Notebook No. 8005, pp. 190-193.

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